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Effect of Temperature on Drug Release from Copoly (L-Lactic Acid/δ-Valerolactone) Microspheres

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Summary: Copoly (L-lactic acid/δ-valerolactone) microspheres (PLV-MS) containing *p*-hydroxybenzoic acid ester (ethyl (PE), *n*-propyl (PP), *n*-butyl (PB)) or 3',5'-dibutyryl-2'-deoxy-5-fluorouridine (C4-FUdR) were prepared. The effect of temperature on the drug release rate constant (KH) and the release mechanism from PLV-MS were investigated *in vitro*. All KH values observed for PLV-MS increased with a rise in temperature. In the relationship between log KH and the reciprocal of absolute temperature, relatively hydrophilic PE and PP gave the liner profiles throughout the temperature range examined; lipophilic PB and C4-FUdR exhibited a break point at a temperature near glass transition temperature (Tg) of copolymer in the profiles. The relationship between the initial content of the drugs in the PLV-MS and KH suggested that PB was contained mainly in the suspended state in PLV-MS and that the release of PB from PLV-MS occurs by the dissolution and diffusion of PB in the copolymer phase, which formed PLV-MS. On the other hand, KH observed in PLV-MS containing PE or PP and the diffusion coefficient of PE or PP in the PLV-MS were independent on the initial content of drugs. Therefore we estimated that PE or PP was contained in the completely dissolved state in PLV-MS. These results suggest that temperature dependency of the release rate from PLV-MS is governed by the states of contained drug in PLV-MS.

Keywords: copoly (L-lactic acid/δ-valerolactone); microsphere; release mechanism; temperature

Introduction

Various approaches to developing fine particles (for example, microspheres and nanospheres) as the drug carrier have been studied. Once these carriers are delivered to the target site in the body, the drug should be released at an appropriate rate from the carrier. We reported previously on the release behavior of a lipophilic prodrug, 5-fluoro-2'-deoxyuridine (3',5'-diacyl-5-fluoro-2'-deoxyuridine), from poly (L-lactic acid) microspheres (PLA-MS) *in vitro*.[1,2] The prodrug release from the PLA-MS was enhanced in the presence of an esterase, an enzyme catalyzing the hydrolysis of the prodrug to hydrophilic 5-fluoro-2'-deoxyuridine (FUdR). Albumin, which increased the apparent solubility of the prodrug itself in the release medium, also accelerated the release rate. These results indicated that the drug release proceeded mainly by diffusion of the drug in the aqueous medium, which had penetrated into the capillaries or cracks formed within PLA-MS because of a crystalline nature of PLA. This characteristic of the drug release from PLA-MS suggested that the prediction of the drug release rate *in vivo* from *in vitro* may be difficult. When microspheres are prepared by using a polymer that is in a rubbery state at body temperature, the drug release would be controlled by the diffusion of the drug in the polymer matrix itself, not in the aqueous channels or cracks. Therefore, these

microspheres could be useful to obtain information about the difference in the drug release property between *in vitro* and *in vivo*. Copoly (L-lactic acid/δ-valerolactone) (PLV), an average molecular weight (Mw) of which is about 10,000, has glass transition temperature (Tg) near body temperature.[3] Such a copolymer is in a glassy state at temperatures below Tg and in a rubbery state at temperatures above Tg. By the use of PLV having Tg below body temperature to prepare PLV microspheres (PLV-MS), it may be possible that the drug release from PLV-MS is suppressed by a glassy state of PLV at low temperature during storage, and the drug release is forwarded by the change of PLV phase after administration of PLV-MS *in vivo*. By the use of PLV having Tg above body temperature, a thermosensitive polymer carrier (on-off drug release system) applicable, for example, to hyperthermic treatment in cancer therapy may be designed. Although many thermosensitive carriers such as hydrogel and liposomes, have been investigated,[4–8] reports concerning an approach by microspheres employing biodegradable polymer are limited.[9,10]

In the present study, *p*-hydroxybenzoic acid ethyl ester (PE), *p*-hydroxybenzoic acid *n*-propyl ester (PP), *p*-hydroxybenzoic acid *n*-butyl ester (PB), and 3',5'-dibutyryl-FUdR (C4-FUdR) were used as model drugs to prepare PLV-MS. PE, PP, and PB were used to examine relationship between the physicochemical property and the drug release, and C4-FUdR was used as a model antitumor drug. The effect of temperature on the drug release rate from PLV-MS, the relationship between the drug release rate and Tg, and the mechanism of the drug release have been investigated.

Experimental

Materials

PLV (L-lactic acid/δ-valerolactone, 90/10, mol%, Mw = 11,500, Tg = 25°C, all data given by the supplier) was purchased from Taki Chemical Co. (Hyogo, Japan). PE, PP, and PB were purchased from Tokyo Kasei Kogyo Co. (Tokyo). FUdR was a gift from Yamasa Shoyu Co. (Chiba Pref., Japan). C4-FUdR was synthesized by the procedure described by Nishizawa *et al.*[11] The compound was identified by elemental analysis, nuclear magnetic resonance (NMR), and mass spectrum (MS). The compound was more than 98% pure, as shown by one major peak by high-performance liquid chromatography (HPLC). Porcine liver esterase suspension was purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). The esterase suspension (2,600 units/ml) in 3.2 M $(NH_4)_2SO_4$ solution was diluted with 0.01 M phosphate buffer (pH 7.4) to give a final concentration of 100 units/ml; the resultant solution was then filtered through a membrane filter (0.45 μm), Toyo Roshi Co., Ltd., (Tokyo). The esterase preparation was stored at 4°C until use. All other chemicals were commercial reagent grade products.

Measurement of partition coefficients

The apparent partition coefficients of the drugs were determined in a methylene chloride/water system. A weighed amount of each drug was dissolved in methylene chloride (4 ml). Water (4 ml) was added to the organic solution, which was shaken for 10 min. The mixture was permitted to stand for 48 h at 4°C. Then the drug concentration in the aqueous layer was determined by HPLC.

Preparation of PLV-MS

PLV-MS containing PE, PP, and PB were prepared by the solvent-evaporation method similar to that reported previously.[2] The weighed amount of each drug (5–20 mg) and PLV (100 mg) was dissolved in methylene chloride (2 ml). The organic solution was then dispersed in 1.5% polyvinyl alcohol solution (90 ml) under stirring at 500 rpm by means of a three-bladed propeller. The stirring was continued for 3 h at 4°C. In the case of PLV-MS containing C4-FUdR, C4-FUdR (10 mg) and PLV (100 mg) were dissolved in methyl-

ene chloride (4 ml). The organic solution was then dispersed in 1.5% polyvinyl alcohol solution (90 ml) under vigorous stirring with ultra sonicator (150 W) in a cooling bath. The stirring was continued for 3 min to make an o/w emulsion, and the emulsion was then stirred at 2,000 rpm with homogenizer for 6 h at 4°C. The hardened PLV-MS were washed with water and freeze-dried.

Determination of sizes of PLV-MS

The median diameters of PLV-MS were measured by a particle analyzer (SALD-1100, Shimadzu, Kyoto, Japan).

Determination of drug contents in PLV-MS

In the case of PLV-MS containing PE, PP, and PB, a weighed amount of PLV-MS containing each drug was dissolved in chloroform, and the drug concentration was determined spectrophotometrically at 265 nm. In the case of PLV-MS containing C4-FUdR, a weighed amount of PLV-MS was dissolved in acetone, and the solution was diluted with acetonitrile. The drug concentration of the solution was determined by HPLC. The drug contents of PLV-MS (weight ratio, drug/PLV-MS, %) were then calculated.

Effect of temperature on drug release rate from PLV-MS in vitro

To study the effect of temperature on the drug release rate from PLV-MS *in vitro*, release studies were carried out at various temperatures (17–42°C) by the following method. The weighed amount of PLV-MS in a flask was suspended in water. The flask was immersed in a shaker bath maintained at indicated temperature and shaken horizontally. At fixed time intervals, an aliquot of the solution was withdrawn, and an appropriate volume of fresh medium was added to the release medium to maintain a sink condition, in which the concentration of each drug in the medium was less than 5% of its solubility. The amount of drug released was calculated after HPLC determination of the solution.

The apparent release rate constants (KH) were obtained by the method described by Y. Aso *et al.*[9] Linear Higuchi plots (percent of drug released *versus* square root of time) for the data were made, and KH were then calculated from the slope of the straight lines.

Effect of pH and esterase on drug release from PLV-MS in vitro

To study the effect of pH and esterase on drug release from PLV-MS containing PB *in vitro*, release studies were carried out at 37°C in the sameway as those described above. Water, 0.01 M phosphate buffer solution (pH 7.4), 0.02 M disodium hydrogen phosphate solution (pH 8.5), and 0.01 M phosphate buffer (pH 7.4) containing 2.3 unit/ml porcine liver esterase were used as a release medium.

HPLC assay

The HPLC system (LC-3A, Shimadzu) equipped with a variable wavelength detector (SPD-2A, Shimadzu) was used. The stationary phase was LiChrospher RE-18e (5 μm) packed in a stainless steel column (4.0 × 250 mm, Merck). The mobile phases were water : acetonitrile (60 : 40) for PE, (50 : 50) for PP, (40 : 60) for PB, and 0.1% acetic acid solution : acetonitrile (40 : 60) for C4-FUdR, with a flow rate of 1.0 ml/min. The wavelengths were 253 nm for PE, PP, and PB and 270 nm for C4-FUdR.

Results and Discussion

Preparation of PLV-MS

PE, PP, and PB were used as model drugs to prepare PLV-MS (Table I). The drug contents in PLV-MS containing PE, PP, or PB increased when the initial loading amount of

TABLE I. Apparent Partition Coefficients of Drugs, Amounts of Drug and PLV Used for Preparation of MS, Drug Contents in MS, and Median Diameter of MS

Drug	log *P*	Amount for preparation		Drug content[a] in PLV-MS	Median diameter[a]
		Drug (mg)	PLV (mg)	(%)	(μm)
PE	1.33	7	100	0.57 ± 0.02	53.16 ± 3.47
PE		14	100	1.15 ± 0.10	52.40 ± 2.63
PE		20	100	1.71 ± 0.11	55.89 ± 5.17
PP	1.86	7	100	1.30 ± 0.11	54.14 ± 3.74
PP		14	100	2.59 ± 0.20	49.14 ± 3.12
PP		20	100	3.71 ± 0.30	48.57 ± 1.94
PB	2.45	7	100	1.65 ± 0.36	55.34 ± 3.17
PB		14	100	4.02 ± 0.77	49.46 ± 3.96
PB		20	100	7.29 ± 0.79	50.46 ± 2.60
C4-FUdR	2.55	10	100	2.04[b]	2.83[b]

a) Mean ± S.D.; $n = 3$. *b*) $n = 1$.

each drug was increased. When the same amount of each drug was used, the drug contents increased as the lipophilicity of the drug increased. Partitioning of the drug between the organic phase and aqueous phase during the solvent-evaporation process would greatly affect the final content of the drug in the PLV-MS. Therefore the highly lipophilic drugs were retained in the polymer phase. In preliminary experiments, the incorporation efficiency of FUdR in PLV-MS was very low (1.6%) because FUdR was a hydrophilic drug. On the other hand, the incorporation efficiency of C4-FUdR in PLV-MS was 22.4%. Therefore the chemical modification of FUdR is helpful to improve the efficiency of drug incorporation into PLV-MS in the solvent-evaporation method.

The diameter of PLV-MS containing C4-FUdR was 2.83 μm (median diameter). When compared with larger microspheres, these small microspheres may be practically advantageous in applying to various routes of administration. However, since particle size control was difficult in the preparation process, PLV-MS containing PE, PP, or PB were prepared without ultrasonication, and the median diameter of PLV-MS was about 50 μm. The diameters of PLV-MS prepared by the simplified method were independent on drug species (*i.e.*, PE, PP, or PB) and drug contents.

Although the contained drug may affect the Tg values of polymers, we could not detect the Tg values of PLV-MS on DSC analysis.

Effect of temperature on drug release rate from PLV-MS

To study the effect of temperature on drug release from PLV-MS (shown in Table I) *in vitro*, the release studies were carried out at indicated temperatures (42, 37, 32, 27, 22, 17°C) around Tg for 6 h. Since the shape and particle size of PLV-MS were not changed after the release studies and the release profiles showed no abrupt change, the degradation or agglomeration of PLV-MS could be negligible during the release study. To compare the drug release rate from each PLV-MS, the apparent release rate constants (KH) at the initial release were obtained from Linear Higuchi plots, according to the method described by Y. Aso *et al.*[9] Log KH *versus* the reciprocal of absolute temperature (1/T) were plotted in Fig. 1. All release rates from PLV-MS increased steeply with the rise in temperature. Relatively hydrophilic PE and PP gave the liner profiles throughout the temperature range examined, and the break point was observed in the profiles for relatively lipophilic PB and C4-FUdR. Although the Tg values of PLV-MS were unknown, the break points were suggesting that these values could be similar to that of PLV without drugs. Especially in the case of C4-FUdR, the release rate increased rapidly when the temperature rose to 27°C (above Tg of PLV) from 22°C (below Tg of PLV). Therefore if a PLV sample having Tg above body temperature, for example 40°C, is available as the

material for microspheres, it may be possible to develop an on-off drug release system because the drug release is initiated by the heating of a target site where the microspheres were delivered. As a general rule of polymer, the movement of polymer chains rapidly increases with the rise in temperature, and at a temperature above Tg, the permeability of gas molecules suddenly increases with a polymer drop in viscosity. It is also known that temperature dependence of the drug transport in the polymer is variously changed not only by the size of drug molecule, which is related to the diffusional resistance, but also by the difference in mechanism of the drug transport in each polymer phase at temperatures above and below Tg. We also think that the mechanism of the drug release from PLV-MS was changed by the change in the polymer phase at a temperature across Tg; however, the break point was not always observed in the profiles (Fig. 1). The existence of a break point may depend on the physicochemical properties of each drug.

The mechanism of drug release from PLV-MS at a temperature above Tg

The attempt to clarify the mechanism of drug release from PLV-MS is very important not only on the design of PLV-MS, but also on the understanding of the difference of temperature dependency between the drugs.

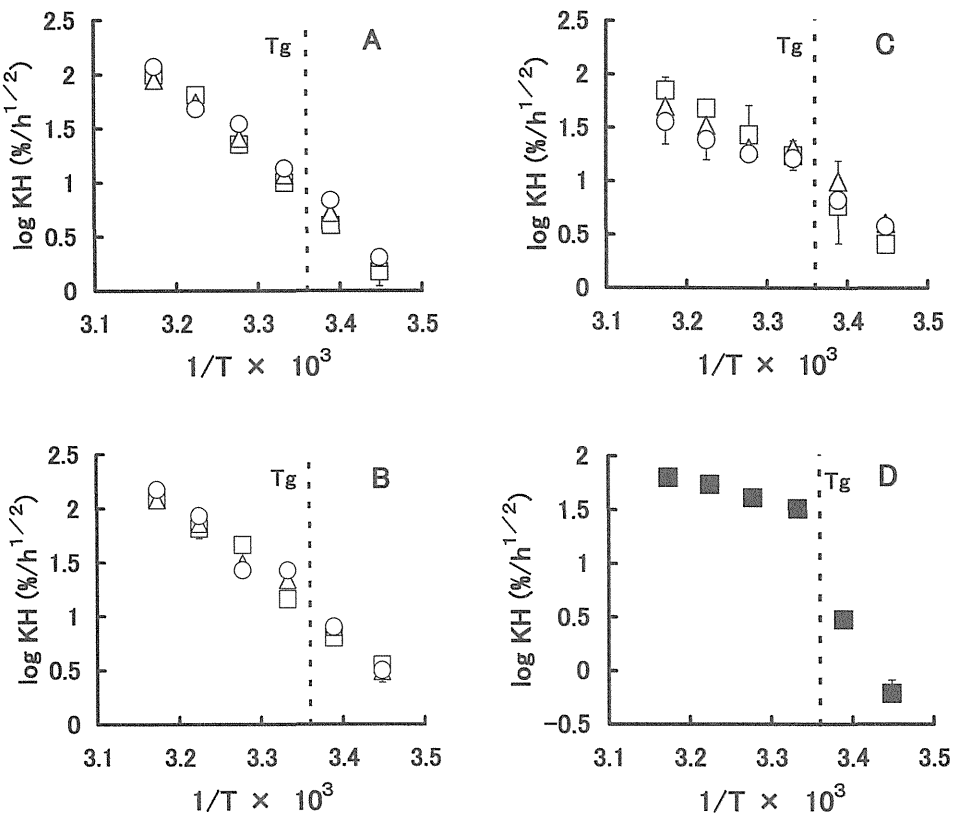

Fig. 1. Effect of Temperature on the Apparent Release Rate Constant (KH) of PLV-MS
A: KH of PLV-MS containing PE, B: KH of PLV-MS containing PP, C: KH of PLV-MS containing PB, D: KH of PLV-MS containing C4-FUdR. A, PE content; □, 0.57%; △, 1.15%; ○, 1.17%; B, PP content; □, 1.30%; △, 2.50%; ○, 2.59%; C, PB content; □, 1.65%; △, 4.02%; ○, 7.29%; D, C4-FUdR content; ■, 20.4% (Median diameter = 2.83 μm). The data are expressed as the mean ± S.D. ($n = 3$). Interrupted lines show Tg of PLV.

The release rate of a drug from a homogeneous spherical matrix has been described by Higuchi[12] and Baker and Lonsdale.[13] When the contained drug is dissolved completely in the matrix and the drug release from the matrix occurs by diffusion in the matrix, the drug release from the matrix is defined by the following equation.

$$F = 6(Dt/r^2\pi)^{1/2} - 3Dt/r^2 \ (F < 0.4) \quad (1)$$

where F and D are the ratio of the drug released at time t and the diffusion coefficient of the drug in the matrix, and r is the radius of the spherical matrix. On the other hand, if the contained drug is suspended in the matrix, which is in excess of its solubility in the matrix, and the drug release from the matrix occurs by the dissolution and subsequent diffusion of drug in the matrix, it is defined by the following equation.

$$(3/2)\,[1-(1-F)^{2/3}]-F = (3D\mathrm{Cms}/r^2\mathrm{Co})t \quad (2)$$

where Cms and Co are the solubility of the drug in the matrix and the concentration of the drug in the matrix at $t = 0$, respectively. A comparison of the drug release profiles from PLV-MS with various Co values can be a convenient method to judge the release mechanism. If the mechanism of the drug release from PLV-MS follows eq. 1, the drug release rate (change of F with t) is independent of the initial drug contents in PLV-MS. If it follows eq. 2, the drug release rate from PLV-MS decreases as the initial drug content in PLV-MS is increased because Co is in the denominator of the right side (eq. 2).

On the basis of these theories, KH values at 42°C above Tg of PLV-MS containing PE, PP, or PB (the median diameter of each PLV-MS is approximately 50 μm) are plotted against the drug content of each PLV-MS (Fig. 2). Only in the case of PLV-MS containing PB, the greater the drug contents in PLV-MS, the smaller the value of KH became. From this result, we assumed that the mechanism of the release from PLV-MS containing PB is described by eq. 2 rather than by eq. 1. If the assumption is true, the plot of the left side of eq. 2 *versus* t should be a straight line because $3D\mathrm{Cms}/r^2\mathrm{Co}$ is constant in the right side of eq. 2. Therefore the values of the left side of eq. 2 (*i.e.*, $3/2[1-(1-F)^{2/3}]-F$) were calculated from data of the cumulative amount of PB released from PLV-MS in release studies at 42°C. The values are then plotted *versus* time (Fig. 3). A straight line was found in each drug content of PLV-MS. Each slope (KB) then substituted for $3D\mathrm{Cms}/r^2\mathrm{Co}$ was plotted *versus* the reciprocal of the drug content (Fig. 4). Figure 4 shows a linear relationship as indicated by eq. 2. These results suggested that PB was contained mostly in a suspended state in PLV-MS and released from PLV-MS by the dissolution and subsequent diffusion of PB in the homogeneous copolymer phase that formed PLV-MS, and it seemed reasonable for the release of PB from PLV-MS to be expressed by eq. 2. On the other hand, since there was no clear tendency that the release rate of PE or PP from PLV-MS was dependent on the drug contents in PLV-MS, we estimated that PE and PP were completely dissolved in PLV-MS. Because we think PE or PP is completely dissolved in PLV-MS and the release from PLV-MS occurs by diffusion

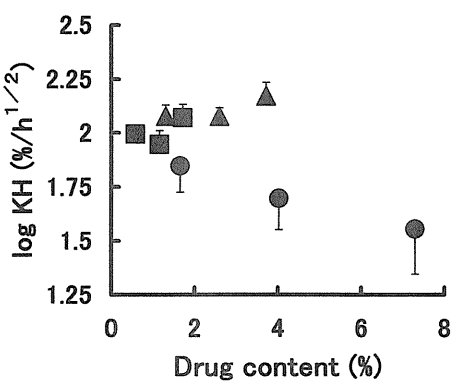

Fig. 2. Effect of Drug Content in PLV-MS on KH at 42°C
■, PE; ▲, PP; ●, PB. The data are expressed as the mean ± S.D. ($n = 3$).

of the drug in the homogeneous copolymer phase that forms PLV-MS, it is reasonable to assume that eq. 1 is applicable to describe the release of PE and PP from PLV-MS. Therefore eq. 3 was obtained by differentiating eq. 1, and the diffusion coefficient (D) was then obtained by eq. 3.

$$dF/dt = 3(D/r^2\pi t)^{1/2} - 3D/r^2 \quad (3)$$

The release rate (dF/dt) obtained by release studies was plotted *versus* $1/t^{1/2}$, and the slopes were then calculated from the plot. Since the slope equals $3(D/r^2\pi)^{1/2}$, the diffusion coefficient (D) was calculated, where (the median diameter/2) of PLV-MS was substituted

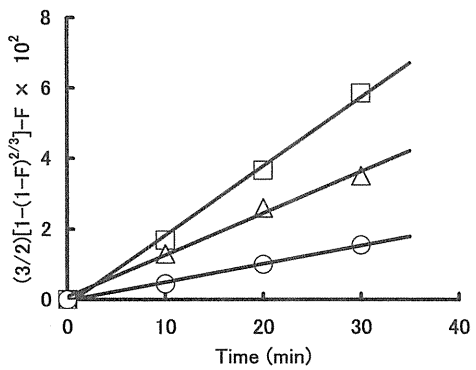

Fig. 3. Relationship between Calculated Values of $(3/2)[1-(1-F)^{2/3}]-F$ and Time for the PB Release from PLV-MS at 42°C
PB content: □, 1.7%; △, 3.0%; ○, 6.1%.

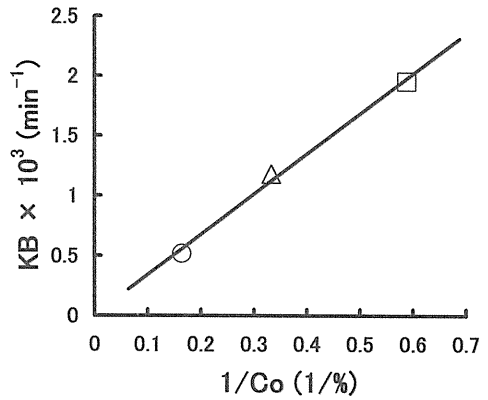

Fig. 4. Relationship between KB and 1/Co
PB content: □, 1.7%; △, 3.0%; ○, 6.1%.

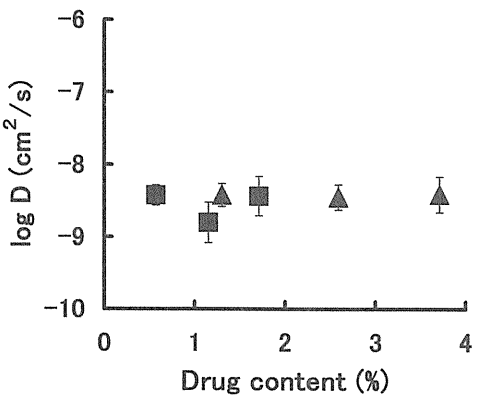

Fig. 5. Effect of Drug Content in PLV-MS on the Diffusion Coefficient (D) of the Drugs at 42°C
■, PE; ▲, PP. The data ara expressed as the mean ± S.D. ($n = 3$).

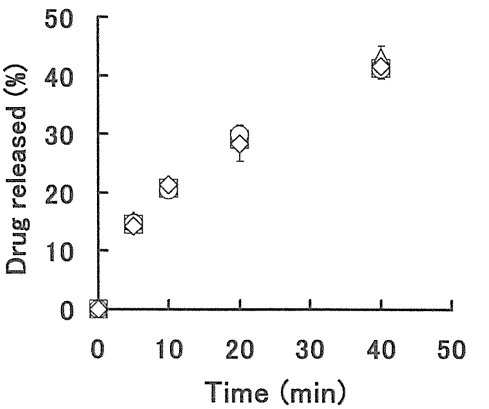

Fig. 6. Effect of Esterase and pH Condition on the Drug Release from PNLV-MS Containing PB at 37°C
□, distilled water; ◇, 0.01 M phosphate buffer (pH 7.4); △, buffer containg 2.3 untits/ml esterase; ○, 0.02 M disodium hydrogenphosphate solution (pH 8.5). The data are expressed as the mean ± S.D. ($n = 3$). In the case of buffer containing esterase, the percentages of release were calculated from the total amount of drug released (PB plus *p*-hydroxybenzoic acid).

by r. The relationship between the obtained D and the drug contents in PLV-MS is shown in Fig. 5. The values of D were approximately constant irrespective of the drug and drug content. From these results it can be estimated that the mechanism of drug release from PLV-MS becomes different in the different states of dissolved drug in PLV-MS, and the state of dissolved drug in PLV-MS is dependent on the lipophilicity of the drugs. To study the effect of PLV on the melting point of each drug, the depression of the melting point was measured by a differential scanning calorimeter. The depression of the melting point of PE and PP was 20.3°C and 11.6°C, respectively, and that of PB was only 2.6°C. This also supports our consideration described above. Figure 1 shows that the release rates of PB from PLV-MS slightly increase with temperature in the temperature range above Tg of PLV. This may be explained that the releases of PB increase by the gentle increase in drug solubility in PLV-MS at a higher temperature. On the other hand, the release rates of PE and PP increase with an increase in D, which was favored by decreased viscosity at a higher temperature. C4-FUdR may be suspended in PLV-MS like PB because the lipophilicity of C4-FUdR is nearly equal to that of PB, and the profile of C4-FUdR in Fig. 1 was similar to that of PB.

We reported previously that the drug release from PLA-MS is largely influenced by albumin and esterase in release medium because the drug release from PLA-MS would be driven by dissolution and diffusion of the drug in an aqueous medium that had penetrated through drug-presented capillaries or cracks formed within PLA-MS.[1,2] On the other hand, considering the case of the drug release from PLV-MS at a temperature above Tg, since we thought that the diffusion of the drug in the copolymer phase forming PLV-MS is a rate-determining step, we expected that the drug release from PLV-MS is not influenced appreciably by various body fluid components. Therefore to clarify the effect of pH and esterase on the drug release from PLV-MS, the release studies from PLV-MS containing PB were carried out (Fig. 6). On the addition of the esterase, PB was converted to *p*-hydroxybenzoic acid; the percent of release at an indicated time was calculated by the total amount of the drug released, that is, PB plus *p*-hydroxybenzoic acid. The results differed from the case of PLA-MS. It was shown that the drug release from PLV-MS was affected neither by high pH condition, which increases the solubility of the drug in the release medium, nor by esterase, an enzyme catalyzing the hydrolysis of PB to hydrophilic *p*-hydroxybenzoic acid. This result also supports our opinion that the diffusion of the drug in the copolymer phase that formed PLV-MS is a rate-determining step in the drug release process for PLV-MS.

In conclusion, the microspheres were prepared by use of PLV (Tg = 25°C), which is in a rubbery state at body temperature. The drug release rates from PLV-MS were increased with a rise in temperature. Especially in the case of C4-FUdR, the release rate increased steeply when temperature exceeded Tg of PLV. Therefore if PLV having an optimum Tg can be used as the material of the microspheres, it is possible to develop an on-off release system. On the other hand, since it was suggested that the drug release from PLV-MS at a temperature above Tg is controlled by the diffusion of the drug in the copolymer phase that formed PLV-MS, it is expected that a comparable drug release rate can be obtained both *in vitro* and *in vivo* until the biodegradation of PLV becomes remarkable.

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